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The response of soil arthropods to canopies of black walnuts

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With one figure

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1. Introduction

The response of soil arthropods to single-habitat factors such as soil organic matter or particle size has been infrequently demonstrated. Most soil arthropod faunal studies are samples of species present in different habitats. In such studies, species differences in soil arthropods from habitat to habitat are due to the interaction of microclimate, vegetation, and soil properties. The classic example of this approach is the study of WEIS-FOGH (1948). A recent example is the study of DAVIS (1963) who studied correlated change in soil arthropod faunas with changes in vegetation and soil properties occurring during grassland reclamation. In addition to faunal surveys, there are field studies identifying some of the causal factors affecting soil arthropod abundance. For example, the contrasting effects of three plant species on soil arthropod density were demonstrated by MACFADYEN (1952) who sampled from intermixed tussocks of three grass and sedge species. The effect of climate on collembolan distribution was demonstrated by STEBAEVA (1967) who made reciprocal exchanges of soil blocks between plant communities.

This study extends the approaches of MACFADYEN (1952) and STEBAEVA (1967) by demonstrating the causal effect of one plant species on soil arthropods in the forest floor of an oak-hickory forest. Black walnut trees (*Juglans nigra* L.) were chosen for study because they are known to release one or more substances which affect the organisms around them (THOMPSON 1957). Collections were planned to answer three questions. First, are there differences in soil arthropod species numbers and density under black walnut canopies in relation to the rest of the forest? Second, if there is a black walnut canopy effect, can soil block transfer be used to show that the canopy effect is due to a factor such as a toxin, which disappears with time? Third, does soil block transfer itself change soil arthropod density or species numbers?

2. Materials and methods

This study was carried out in a mature upland woods. The predominant tree species in the area were red oak (*Quercus rubra* L.) and white oak (*Quercus alba* L.). The wood has not been disturbed and is part of the Cook County (Illinois) Forest Preserve (Bemis Woods North: T39N, R12E, Sec. 31). The soil, a Mollisol in the Plano series, was developed under prairie vegetation on medium- to moderately-textured glacial outwash and is well drained (FEHRENBACHER, WALKER, and WASCHER 1972).

The area around seven black walnut (*Juglans nigra* L.) trees with DBH between 32 cm and 53 cm was chosen for sampling. In fall prior to spring sampling, two pairs of soil blocks associated with each of the seven trees were moved (Figure 1). The soil blocks were moved on 24, 29 November and on 5 December 1972. A soil block from under each black walnut canopy was exchanged with a soil block taken from outside the canopy of that tree. A second pair of soil blocks was simply removed from the ground and rotated 180°: one under a black walnut canopy, one outside the canopy.

The location of soil blocks around any black walnut tree was determined by choosing two sets of random numbers: the first three digits determined the angle from north, the second three digits determined the distance from the tree. Plots located outside black walnut canopies were placed in the direction that was chosen from the first set of random numbers; all were 17 meters from the black walnut trunk.

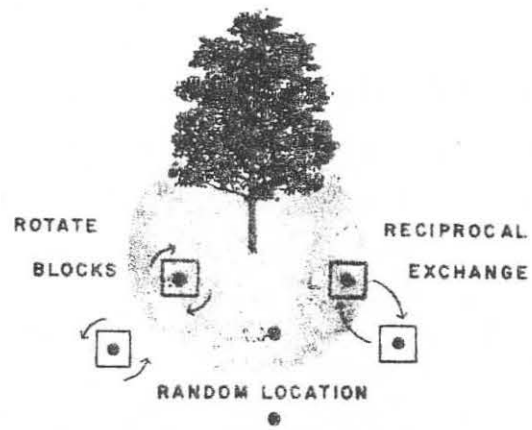

Fig. 1. Diagram showing the location of the 6 collections made around each black walnut tree. Solid circles indicate locations of soil core collections under (shaded) and outside of black walnut canopy.

Soil cores were collected on 1 April 1973; the soil had completely thawed at that time. Soil cores were removed from each of the seven sets of four soil blocks which had been moved the previous fall. In addition, one soil core was randomly collected from unmoved soil under each black walnut canopy and one from outside each canopy, giving a total of 42 samples.

Each of the soil blocks was sampled at its center to avoid any edge effect. The soil blocks were 60 cm × 61 cm and 9 cm deep. Soil cores were collected with a steel corer made to take six-ounce cardboard orange juice cans as core liners. The cores were 53 mm in diameter, 9 cm deep. All samples were collected and placed in an extractor on the same day. Soil cores were divided into 3-cm sections, and each section inverted in the extractor. Microarthropods were extracted into dishes of picric acid with a high gradient extractor (Lussenhop 1971). Specimens were filtered from the picric acid, cleared in lactic acid, and hand-mounted in Berlese's medium on large microscope cover slips. The arthropods from each 3-cm core section were then identified using the phase contrast optics of a compound microscope. The numbers of individuals present in each 3 cm core section were recorded separately on punch cards.

The results were analyzed using a two-way analysis of variance, using a fixed effects model. The treatment sum of squares can be divided into five orthogonal contrasts (Table 1), which can be grouped to answer questions posed in the Introduction. Data presented in the analyses of variance have been tested for homogeneity of variance with Bartlet's test (Brownlee 1960); when analyses of variance for individual species were calculated, the square root transformation was used.

Table 1. Partition of treatment sum of squares into orthogonal contrasts

Contrast test Effect of:	Collections from under Black Walnut canopy			Collections from outside Black Walnut canopy		
	Control	Sample	Transfer	Control	Sample	Transfer
Black Walnut canopy	+1	+1	+1	−1	−1	−1
Soil block movement	+1	−1	0	0	0	0
	0	0	0	+1	−1	0
Black Walnut toxin	+1	+1	−2	0	0	0
	0	0	0	+1	+1	−2

Column headings indicate collections from rotated soil blocks (control), reciprocally exchanged soil blocks (transfer) or from unmoved soil (sample).

After soil arthropod extraction, *p*H and organic carbon of the soil cores were determined. Only soil cores which had not been transferred (controls and samples) were used. The *p*H of soil cores from under the canopy and outside the canopy of each black walnut was measured. Subsamples from the two collections associated with each tree were composited and used to determine soil organic carbon (Mebius 1960).

3. Results

3.0. General remarks

The black walnut trees used in this study were old enough to have affected the soil under their canopies. The average *p*H of soil from which arthropods were extracted was 5.4 outside black walnut canopies, compared with the greater average *p*H, 6.3 under the canopy ($.001 < P < .005$). Soil organic carbon was also significantly greater under black walnut canopies (3.5 %) than outside the canopies (3.2 %; $.001 < P < .005$).

3.1. Canopy effect

In April, soil cores collected under black walnut trees had significantly fewer species and significantly fewer individuals when contrasted with soil cores collected outside black walnut canopies (Table 2). The average number of soil arthropod species under black walnut canopies was 1.5/cm² in contrast to 2.3/cm² outside the canopy. Similarly, soil arthropod density under black walnut canopies was lower relative to density outside the canopy (3.6 individuals/cm² *versus* 6.8 individuals/cm²; Table 2). The reductions in species number and density are primarily due to significant loss of oribatid mites and collembola (Table 2). The other subclasses of mites (Parasitiformes, Prostigmata, and Acaridae) show no significant response to black walnut canopies.

Table 2. Response of soil arthropods to black walnut (*Juglans nigra* L.) canopies, soil block transfer, and to properties of soil under black walnut canopies

Source of Variance	d. f.	Mean Squares			
		Species Number	Density of all species	Collembola Density	Oribatei Density
Treatments	5	120.3	1143.9*	97.9	132.6
Canopy	1	427.5**	2672.0**	414.8**	550.1*
Soil block transfer	2	69.0	2.18	9.3	10.6
Reciprocal exchange	2	80.2	452.7	27.8	45.8
Trees	6	85.4	466.1	115.2	86.5
Error	*30*	*72.4*	*414.0*	*59.4*	*109.9*
Total	41				

Significance levels: *, $P < .05$; **, $P < .025$; ***, $P < .01$.

Most of the species which showed a significant canopy response were reduced in numbers under the canopy (Table 3), but the two response groups show no contrast in feeding habits. The density of three predators (*Rhodacarellus* sp., *Rhagidia* sp., and *Pygmotes* sp.) is reduced under black walnut canopies. Three of the oribatids which decreased in number under black walnut canopies are believed by LUXTON (1972) to feed strictly on microflora (*Opiella* sp. 1, *Quadroppia* sp., *Suctobelba* sp.). The collembolan, *Tullbergia* sp., probably feeds on microflora plus plant material (CHRISTIANSEN 1964). Of the species which increase in density under black walnut canopies, the oribatid *Hypochthonius* sp. is believed to feed on microflora (LUXTON 1972), and the collembola probably feed on microflora and dead plant material (*Folsomoides* sp.) as well as algae (*Folsomia* sp. HARDING and STUTTARD 1974).

Is the canopy effect a direct response to black walnut toxins, or is it a response to possible long-term indirect effects of black walnut trees such as increased *p*H or organic matter? In order to answer this question, soil blocks were either exposed to the toxins by transferring them under black walnut canopies, or removed from exposure by transferring them away from canopies. The possible effect of the method itself, soil block transfer, is discussed first.

Table 3. Soil arthropods showing a response to black walnut (*Juglans nigra*) canopies in a 2-way ANOVA

Species with lower density under black walnut canopies	Species with higher density under black walnut canopies
ACARINA	
Mesostigmata	
Rhodacarellus sp. ($P < 0.10$)	
Prostigmata	
Pymotes sp. ($0.01 < P < 0.05$)	
Rhagidia sp. ($P < 0.10$)	
Oribatei	
Autogneta sp. ($P < 0.10$)	*Hypochthonius* sp. ($P < 0.10$)
Opiella sp. 1 ($0.01 < P < 0.05$)	
Quadroppia sp. ($P < 0.10$)	
Suctobelba sp. ($P < 0.10$)	
COLLEMBOLA	
Tulbergia sp. ($P < 0.10$)	*Folsomia* sp. ($P < 0.10$)
	Folsomoides sp. ($P < 0.10$)

3.2. Effects of soil block transfer

Collections were made from soil blocks four months after they had been removed from the ground, rotated 180° and replaced. The contrast between these collections made at the same time from neighboring unmoved soil shows no difference in either soil arthropod species numbers or density (Table 2). Taken singly, no species shows a response to soil block transfer. Because no response to soil transfer was observed, the technique is used to test for a direct, short-term effect of black walnut toxins.

3.3. Toxin effect

Soil blocks transferred under black walnut canopies are subject to leaching of toxins from leaves and branches during the winter. Soil blocks transferred out from under black walnut canopies are no longer subjected to toxins in leachates. If black walnut toxins affect soil arthropods either directly or indirectly through an effect on soil microflora, arthropod numbers in blocks transferred under black walnut canopies should become similar to numbers occurring there. And, soil arthropods in blocks transferred out from under black walnut canopies should recover from effects of the toxins to an extent depending on breakdown and leaching rates, becoming similar to soil arthropods not under black walnut canopies. For this reason, the lack of significant contrast between transferred soil blocks and non-transferred blocks (M. S. for Reciprocal Exchange, Table 2) indicates that toxins have signicantly affected soil arthropods.

4. Discussion

Most of what is known about soil arthropods is based on faunal surveys or on laboratory experimentation. Statistically analyzed manipulative experiments have not often been carried out using soil arthropods because soil effectively buffers arthropods against changes caused by experimental manipulation. The few manipulative experiments which have been successfully carried out and analyzed statistically demonstrate the response of litter rather than soil inhabitants to such relatively drastic treatments as mowing, raking, or burning. For example, PEARSE (1943) demonstrated the response of surface arthropods to raking and burning. Continuing PEARSE's type of study, GILL (1969) replaced the litter in an old field with dacron using a randomized block experiment. GILL's experiment showed that, at least

over a period of 14 months, the microclimatic effect of litter is more important to soil arthropods than its nutritional value. Similarly drastic modification of the soil surface was used by JENSEN et al. (1973) who showed a response of collembola to mowing and raking using a quasi-Latin square design.

It is precisely because of the buffering property of soil that soil block transfer is useful in separating climatic and chemical factors from other environmental factors. STEBAEVA (1967) transferred soil blocks to interchange collembola among forest, grassland, moor, and tundra climates. She exchanged pairs of 20×10 cm by 5 cm deep blocks between habitats and after ten days collected soil cores from the blocks and extracted arthropods from them. In another area, she transferred 20×40 cm by 10 cm deep soil blocks and sampled after 1.5 months. She observed changes in density of litter inhabiting species, but little change among soil inhabiting species. Although some collembolan species in STEBAEVA's study showed change of up to 300% with respect to controls, the significance of such responses is difficult to judge without knowledge of differences between paired samples. From the present study it is clear that over a four-month period in winter, transfer of soil blocks itself causes no significant change in soil arthropod density or species number.

In this study the soil block transfer technique was used to evaluate the effect of black walnut canopies on soil arthropods relative to the rest of the forest floor. Black walnut was chosen because the trees are known to have an effect on plant distribution, soil properties, and the microflora under their canopies. The cause of the effects black walnut trees have on other organisms may be juglone, a naphthaquinone. However, LAGRANGE (1956), working with species of *Bacillis*, distinguished a bacteriocidal and three bacterial inhibiting substances obtained from leaf extracts of the European walnut, *Juglans regia*. For this reason, the canopy effect of black walnuts may be more extensive than laboratory studies of juglone suggest.

BODE (1958), working with *Juglans regia*, has shown that juglone is released from leaves, branches and twigs; roots are not an important source. The persistence of juglone in soil has not been studied, but in this study the arthropod increase in soil blocks transferred out from under canopies suggests that it is broken down in much less than four months. Black walnut trees, perhaps through the action of juglone, raise the *p*H of soils (BROOKS 1941; SMITH 1942); in this study the *p*H increase was 0.9. Soil organic carbon also increases under black walnut trees, SMITH (1942) found that the contrast between organic carbon under black walnut canopies with that in surrounding pasture soil was greater than the contrast caused by black locust (*Robinia pseudoacacia* L.) trees growing in the same Ohio pastures. In Bemis Woods there is a significant increase in soil organic carbon under black walnut canopies compared with the soil of the surrounding forest. Black walnuts change the composition of plant associations under their canopies. For example, BROOKS (1935) recorded the plant species under and adjacent to 300 black walnut canopies. He found that black walnuts favor species in the Leguminosae, Labiatae, and Compositae, and that *Ribes* spp. and *Vitis* spp. characteristically occur under black walnuts.

Juglone kills or inhibits bacterial growth and probably inhibits growth of some fungi as well. The effect of juglone on bacterial growth has been demonstrated in plate cultures by VERONA and FAGIOLI (1954) and by LAGRANGE (1954). Actinomycete growth was not affected by juglone in concentrations of up to 2% (VERONA and FAGIOLI 1954). They also found that concentrations of up to 2% had only a very small effect on the growth of the 24 species of fungi they tested. GRIES (1943) however, found that juglone inhibited growth of two fungal species which he tested.

Juglone slows the feeding activity of such insects as *Scolytus multistriatus* and *Periplaneta americana* (GILBERT et al., 1967; FERKOVICH, et al., 1961). For this reason, it is not clear whether the soil arthropod response observed in this study is the direct effect of juglone or the indirect effect of juglone on the microflora.

The effect of black walnut canopies on soil arthropods observed in this study is similar to the correlations obtained by REEVES (1967). REEVES contrasted oribatids occurring under a stand of black walnuts with oribatids occurring under stands of spruce (*Picea abies*), white

pine (*Pinus strobus*), and mixed hardwoods (*Quercus alba, Q. rubra, Carya ovata*). Ten of the abundant oribatid species, representing more than 2% of individuals collected, are twice as abundant under either black walnut or mixed hardwood stands than under stands of other tree species. Under black walnut canopies, seven of the ten oribatid species increased in density; three species decreased in density. This contrasts somewhat with the present study because in Bemis Woods, one oribatid species increased under black walnuts and three species decreased. There is a limited similarity between taxa which responded in the two studies: in both cases a species of *Hypochthonius* increased under black walnut, and members of the family Oppiidae decreased under black walnuts. Oribatid response to black walnut does not appear to depend on the type of food ingested; microfloral feeders and plant detrital feeders appear in both response groups.

In this study juglone has been shown to decrease soil arthropod density. Juglone may cause the soil arthropod response directly by slowing arthropod feeding activity, or indirectly by inhibiting microfloral growth. This effect on decomposers has caused accumulation of soil organic matter under black walnut canopies. The present study shows that the effect of plant secondary substances such as juglone may be much more pervasive than WHITTAKER and FEENEY (1971) suggest in their review of allelochemics. Juglone affects not only the higher plants growing around black walnut trees, but also slows the rate of decomposition in soil under black walnuts through its effect on the soil microflora and microfauna.

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6. Summary · Zusammenfassung

Soil cores were collected in April 1974 from under canopies of seven black walnut (*Juglans nigra* L.) trees as well as from outside the canopies of the same seven trees. Under each canopy one soil core was collected from undisturbed soil, one from a soil block which had been rotated in place the previous December, and one from a soil block which had been moved under the canopy the previous December. Similarly, outside the canopy of each tree, one core was collected from unmoved soil, one from a rotated soil block, and one from a soil block which had been moved outside the canopy the previous December.

Soil arthropod species numbers and densities increased significantly in soil blocks transferred out from under black walnut canopies but did not change when soil was transferred in under the canopies. The experimental design enabled us to demonstrate that the response of soil arthropods moved away from canopies was due to a short-lived inhibition due to black walnut canopies, not to other canopy effects. The soil arthropod inhibition by black walnut canopies and the significantly greater percent organic matter in soil under black walnut canopies indicate that these trees slow decomposer activity.

Die Reaktion von Bodenarthropoden auf Einflüsse der Baumkronen von *Juglans nigra*

Bodenausstiche wurden im April 1974 sowohl unterhalb als auch außerhalb des Kronenbereichs von Walnußbäumen (*Juglans nigra* L.) entnommen. Unter jeder Baumkrone wurde je ein Bodenausstich aus dem ungestörten Boden eines Bodenblocks entnommen, der im vorhergehenden Dezember an Ort und Stelle umgewendet wurde, und ein Ausstich aus einem Bodenblock, der zur gleichen Zeit außerhalb des Kronenbereichs entnommen und in normaler Lage unter dem Kronendach gelagert wurde. In entsprechender Weise wurde je eine Probe aus ungestörtem Boden (außerhalb des Kronenbereichs) aus einem am Ort umgewendeten und aus dem Kronenbereich umgelagerten Bodenblock entnommen.

Die Artenzahl und Besatzdichte der Bodenarthropoden stiegen in den aus dem Einflußbereich der Kronen stammenden (außerhalb des Kronenbereichs verlagerten) Bodenblöcken signifikant an. Sie änderten sich jedoch nicht, wenn die Bodenblöcke in entsprechender Weise von außen unter die

Kronendächer umgelagert wurden. Die Versuchsanlage ermöglichte es uns zu zeigen, daß die Reaktion der Bodenarthropoden in Bodenblöcken, die außerhalb des Einflußbereichs der Kronendächer verlagert wurden, durch eine kurzlebige Inhibitionswirkung aus den Baumkronen und nicht durch andere Wirkungen hervorgerufen wurde. Die Behinderung der Aktivitäten von Bodenarthropoden unter Walnußbaumkronen und der signifikant höhere Gehalt an organischer Substanz unter den Baumkronen zeigen, daß diese Bäume den Stoffumsatz verlangsamen.

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